EUROIMMUN Medizinische Labordiagnostika AG

# Test systems for coeliac disease diagnostics

## Comprehensive product portfolio to support the diagnosis and for diet monitoring and risk assessment

- **Anti-Tissue Transglutaminase- + Anti-Gliadin (GAF-3X) ELISA**
- **Anti-Endomysium IIFT + EUROPLUS Anti-Gliadin (GAF-3X) IIFT**
- **EUROLINE Coeliac Disease Profile + EUROLINE Autoimmune Gastrointestinal Diseases**
- **EUROArray HLA-DQ2/DQ8-h Direct**

# Definition and classification of coeliac disease

Coeliac disease (also gluten-sensitive enteropathy, GSE) is a systemic autoimmune disease in which genetic predisposition play a pronounced role. Coeliac disease may affect different organ systems. Its prevalence is estimated to be around 1 %, with experts assuming a large number of undiagnosed cases due to „atypical" or mild symptoms. Coeliac disease is triggered by the consumption of gluten, which makes up about 90 % of the protein contents of many cereal grains. In most cases, the disease manifests as severe inflammation and damage to the mucous membrane of the small intestine (enteropathy). In conjunction with the resulting disturbance of nutrient absorption, a wide range of clinical gastrointestinal and non-gastrointestinal symptoms can develop (including chronic diarrhoea, vomiting, abdominal pain, cramps, short stature, weight loss, delayed puberty, spontaneous abortions, anaemia and osteoporosis). Furthermore, dermatitis herpetiformis (Duhring's disease), a chronic skin rash, can occur.

| Classification of coeliac disease* | Malabsorption | Unspecific symptoms | Enteropathy | Specific antibodies | Genetic predisposition |
|---|---|---|---|---|---|
| **symptomatic** | – | + | + | + | + |
| **classic** | + | +/– | + | + | + |
| **subclinal** | – | – | + | + | + |
| **refractory (only adults)** | + | +/– | + | +/– | + |
| **potential** | – | – | – | + | + |

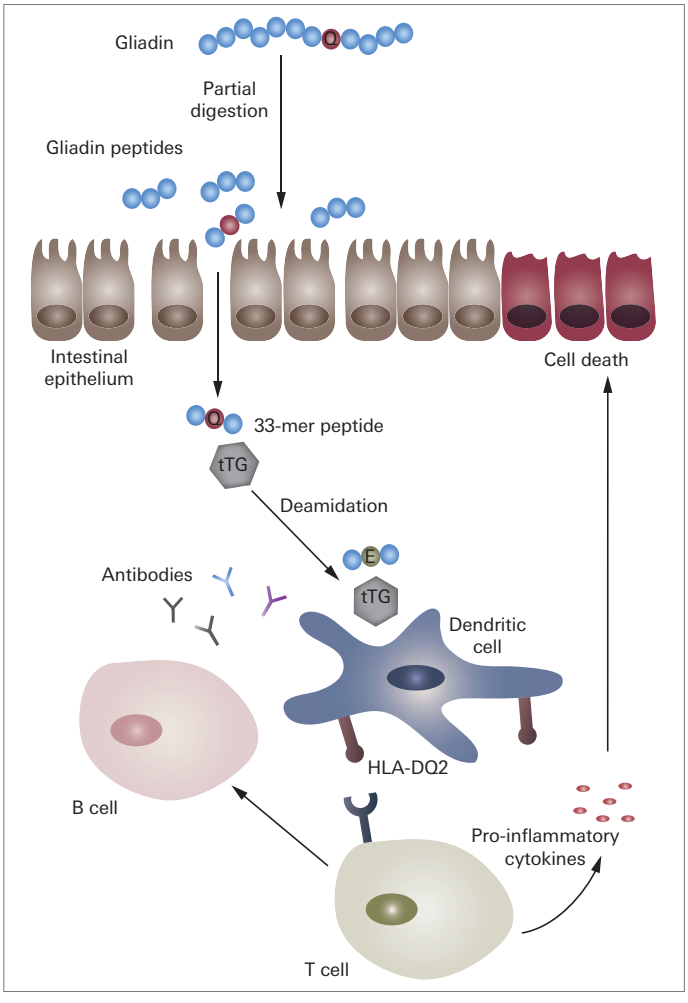
* OSLO classification, Felber J, et al. Updated S2k guideline celiac disease of the German Society for Gastroenterology, Digestive and Metabolic Diseases (DGVS) December 2021 - AWMF register number: 021-021, adapted from Ludvigsson et al, Gut 62:43-52 (2013).

# Pathogenesis of coeliac disease

Both genetic and environmental factors contribute to the development of coeliac disease. Enteropathy, which is characteristic of coeliac disease, is caused by an overreaction of the immune system to gluten components, especially the so-called gliadin.

Gliadin is only partially digested in the small intestine. If there are gaps in the intestinal epithelium, as is typical in patients with coeliac disease, the resulting gliadin fragments (peptides, consisting of 33 amino acids, 33-mer) can pass through the intestinal barrier and reach the connective tissue underneath. There, the enzyme tissue transglutaminase (tTG) modifies (deamidates) the amino acid glutamine (Q) into the amino acid glutamate (E) at certain sites of the gliadin peptides. With the modification, the peptides acquire their immunological effect if the genetic predisposition is present. Especially two genetic variants (DQ2 and DQ8) of the human leukocyte antigen system (HLA system) are associated with the immune reaction. Dendritic cells phagocyte the complex of tissue transglutaminase and deamidated gliadin peptides and, if they express HLA-DQ2 or HLA-DQ8 on their surface, can present it together with the HLA molecules to the T cells of the immune system. The T cells activate the B cells, which then produce antibodies against the deamidated gliadin peptides and against the body-own tissue transglutaminase. In addition, the T cells secrete pro-inflammatory cytokines which cause an inflammatory reaction in the tissue.

The immunological overreaction and the inflammation of the epithelium of the small intestine lead to apoptosis of the enterocytes, atrophy of the villi and widening of the intestinal crypts (hyperplasia). These damages mean that the intestinal mucosa is no longer able to absorb sufficient nutrients from the digested food and to transport them into the bloodstream.

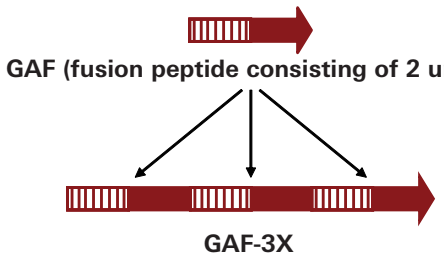


# Overview of parameters and methods for coeliac disease diagnostics

## Serological determination of coeliac disease-specific antibodies

Antibodies against endomysium (EmA) are considered as very specific and sensitive markers for the diagnosis of coeliac disease. They can be detected by IIFT on tissue sections of liver, oesophagus, or intestine (primate). The target antigen of EmA is tissue transglutaminase (tTG). Anti-tTG antibodies can be determined by means of antigen-coated ELISA microplate strips, EUROLINE immunoblots or IIFT using transfected cells. The antibodies associated with coeliac disease also include those against deamidated epitopes of gliadin peptides (anti-DGP antibodies). These can also be detected by ELISA or EUROLINE as well as by means of the monospecific EUROPLUS substrate. Anti-tTG antibodies and EmA of immunoglobulin class A (IgA) are particularly relevant for diagnostics. If a general IgA deficiency is present – a condition which is observed especially often (above average) in patients with coeliac disease – anti-DGP antibodies of immunoglobulin class G (IgG) are considered an important alternative indicator of coeliac disease. Generally, the diagnostic determination of coeliac disease-specific antibodies must be performed under normal, gluten-containing diet since the antibodies disappear with a gluten-free diet.

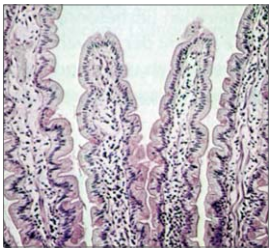
In order to provide specific and sensitive detection of anti-DGP antibodies, EUROIMMUN has developed the antigen substrate gliadin (GAF-3X). This consists of three deamidated gliadin-analogue fusion peptides (GAF) in a row. GAF consists of two synthetic nonapeptides which have proven particularly specific and sensitive for the detection of coeliac disease-specific antibodies amongst the 51 peptides tested.[1] Owing to the reduction of the substrate to two short gliadin peptides, unspecific reactions are prevented and the specificity of the test system is increased. Since GAF is used as a trimer instead of a monomer, also the sensitivity of the test for the detection of the relevant antibodies is optimised.

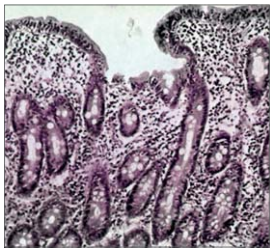


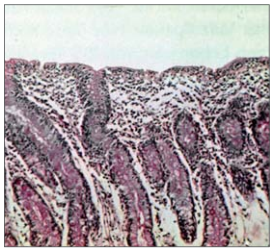

## Histological investigation of a biopsy of the small intestine

Tissue samples are usually taken from different sections of the duodenum, in an endoscopy. The lesions are assessed based on the Marsh criteria, according to the amount of intraepithelial leukocytes (IEL), and the state of the villi and crypts. They are, however, not specific for coeliac disease, but can also develop in other enteropathies.

| Assessment of the histopathological severity according to Marsh |
|---|
| **Type 0:** IEL, villi and intestinal crypts norma |
| **Type 1:** IEL increased, villi and intestinal crypts normal |
| **Type 2*:** IEL increased, intestinal crypts hyperplastic, villi normal |
| **Type 3***: IEL increased, intestinal crypts hyperplastic, villi atrophic |


Marsh type 0


Marsh type 2–3


Marsh type 3

* diagnostically relevant for coeliac disease

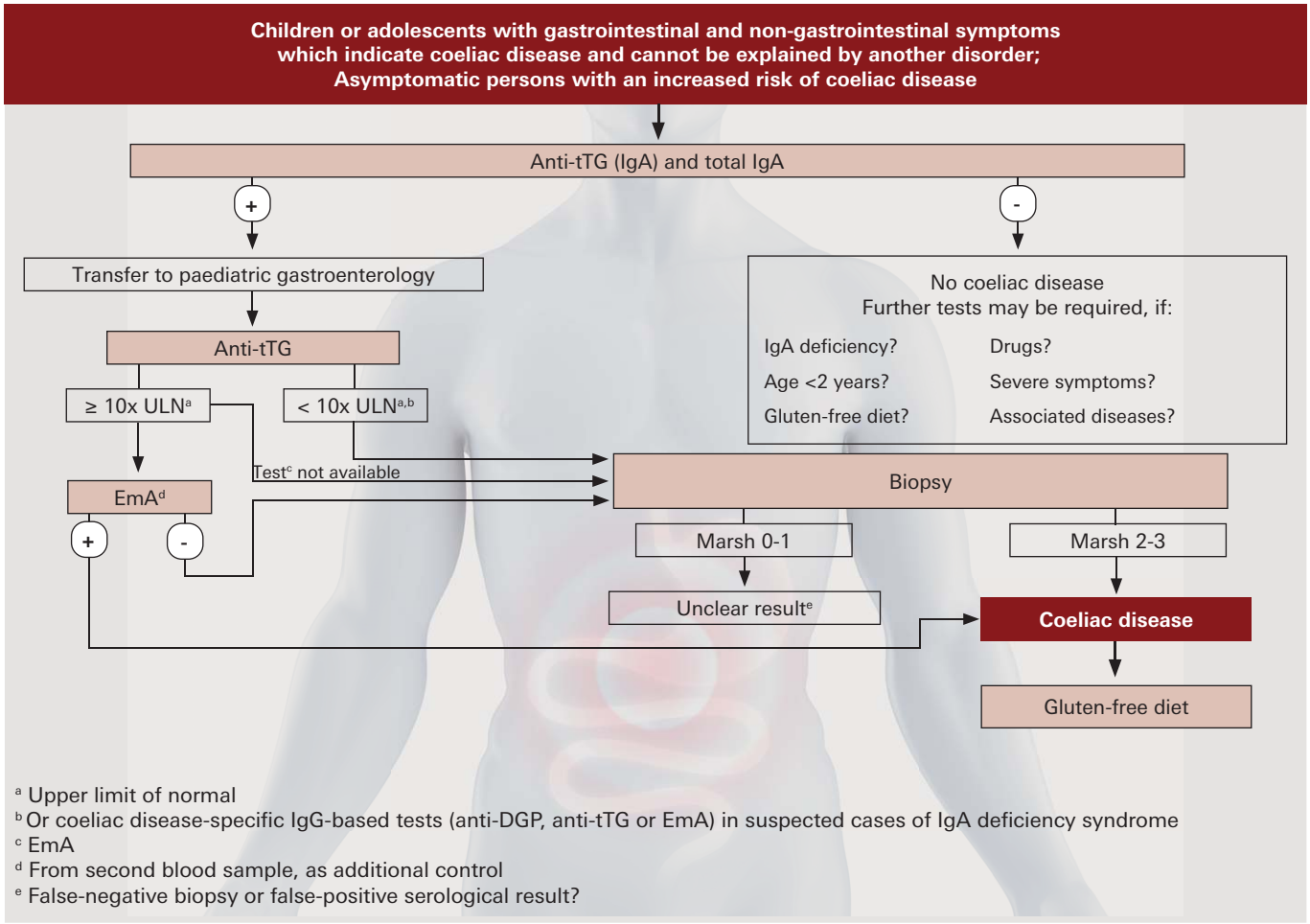
Photos: The German Coeliac Society (DZG)

## Determination of HLA types

The determination of HLA types with molecular genetic test systems such as microarrays can support the diagnosis in patients without high anti-tTG (IgA) titers or patients who have already started a gluten-free diet, as well as patients from certain risk groups (e.g. first-degree relatives or patients with Down syndrome). Since around a third of the healthy population also carries the HLA-DQ2/HLA-DQ8 alleles, their detection can only be used as an indication of coeliac disease and for exclusion diagnostics. If the alleles cannot be detected, the presence of coeliac disease is very unlikely.

# ESPGHAN guidelines for the diagnosis of coeliac disease

According to the guidelines of the European Society of Pediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN) (Husby et al., 2020), patients with relevant symptoms should first be tested for anti-tTG antibodies (IgA) and total IgA antibodies, as these antibodies are particularly specific. If the anti-tTG IgA titer is at least ten times the upper limit of normal (≥ 10x ULN) and a sample taken later is positive for anti-endomysium (EmA) IgA, a biopsy, which is otherwise required to confirm the diagnosis, can be omitted. The guidelines also emphasise the usefulness of coeliac-specific IgG-based tests in other cases, such as tests for the detection of antibodies against deamidated gliadin peptides (DGP). If there is a general IgA deficiency – as often observed in coeliac disease patients – anti-DGP antibodies (IgG) are considered an important alternative indicator of coeliac disease.




[a] Upper limit of normal
[b] Or coeliac disease-specific IgG-based tests (anti-DGP, anti-tTG or EmA) in suspected cases of IgA deficiency syndrome
[c] EmA
[d] From second blood sample, as additional control
[e] False-negative biopsy or false-positive serological result?

# EUROIMMUN test systems for the diagnosis of coeliac disease

## I. ELISA test systems

### Anti-Tissue Transglutaminase ELISA

The serological determination of disease-associated antibodies of immunoglobulin class IgA against tTG is considered the most specific indicator of coeliac disease. Alongside qualitative antibody detection, the Anti-Tissue Transglutaminase ELISA also enables quantitative titer determination. In investigations of different panels the ELISA showed a very high sensitivity of 95.7% at a specificity of 98% for the determination of anti-tTG IgA antibodies.

Alternatively, in suspected cases of IgA-deficiency syndrome, the Anti-Tissue Transglutaminase ELISA (IgG) can be used, which has a very high clinical sensitivity of 99.7%. Owing to the low prevalence of anti-tTG antibodies of the immunoglobulin class IgG, a negative test result does not suffice to exclude coeliac disease. Further tests, e.g. the Anti-Gliadin GAF-3X ELISA (IgG), are required.

| Panel | n | Anti-tTG positive (IgA) |
|---|---|---|
| Coeliac disease (age: 0 - 18 years, bioptically tested) | 183 | 179 (97.8%) |
| Coeliac disease (age: 1 - 54 years, bioptically tested) | 58 | 58 (100.0%) |
| Dermatitis herpetiformis | 36 | 28 (77.8%) |
| **Sensitivity** | **277** | **95.7%** |
| Gastroenteropathies, bioptically negative for coeliac disease | 243 | 12 (4.9%) |
| Chronic inflammatory bowel diseases, bioptically negative for coeliac disease | 55 | 2 (3.6%) |
| Rheumatoid arthritis | 300 | 1 (0.3%) |
| Sjögren's syndrome | 200 | 3 (1.5%) |
| Systemic lupus erythematosus | 150 | 0 (0.0%) |
| Progressive systemic sclerosis | 126 | 4 (3.2%) |
| Bullous pemphigoid | 30 | 1 (3.3%) |
| Linear IgA dermatosis | 22 | 0 (0.0%) |
| **Specificity** | 1126 | 98.0% |

## Anti-Gliadin (GAF-3X) ELISA

Conventional anti-n(ative) gliadin ELISAs are considered to be not very specific since antibodies against native gliadin are also found in healthy individuals. Antibodies against the gliadin fragments used in the Anti-Gliadin (GAF-3X) ELISA, however, are highly specific for coeliac disease. In a retrospective study by Prause et al., the EUROIMMUN test systems Anti-Gliadin (GAF-3X) ELISA, Anti-nGliadin ELISA and Anti-tTG ELISA were compared.[3] Sera from 142 children with active coeliac disease (bioptically confirmed, Marsh 2 or 3) and 160 controls (bioptically confirmed, 19 patients with chronic inflammatory bowel diseases) were investigated for IgA and IgG antibodies. The Anti-Gliadin (GAF-3X) ELISA was generally superior to the Anti-nGliadin ELISA with respect to sensitivity and specificity for both IgA and IgG antibodies. Moreover, the Anti-Gliadin (GAF-3X) ELISA (IgG) was more sensitive and specific than the Anti-Gliadin (GAF-3X) ELISA (IgA) and the Anti-tTG ELISA (IgG) and provided results which were comparable to those of the Anti-tTG ELISA (IgA). A combination of the Anti-Gliadin (GAF-3X) ELISA (IgG) and the Anti-tTG ELISA (IgA) increased the accuracy of the results.

| n = 302 | ELISA | Sensitivity | Specificity |
|---|---|---|---|
| IgA | Anti-nGliadin (native) | 73.9% | 91.9% |
| | Anti-Gliadin (GAF-3X) | 87.3% | 93.1% |
| | Anti-tTG | 95.1% | 98.1% |
| IgG | Anti-nGliadin (nativ) | 88.0% | 80.0% |
| | Anti-Gliadin (GAF-3X) | 95.1% | 94.4% |
| | Anti-tTG | 87.3% | 86.3% |

# III. Indirect immunofluorescence tests (IIFT) for coeliac disease

## Anti-Endomysium IIFT

The Anti-Endomysium IIFT (IgA) is considered an especially specific test for the diagnosis of coeliac disease. Endomysium is a layer of connective tissue which surrounds the muscle cells of the skeletal musculature and the smooth muscle in hollow organs and blood vessels. Standard substrates for the detection of EmA in IIFT are tissue sections of primate liver, primate oesophagus or primate small intestine. The substrates are offered in the form of miniaturised BIOCHIPs which can be variably combined on one test field (BIOCHIP Mosaic).

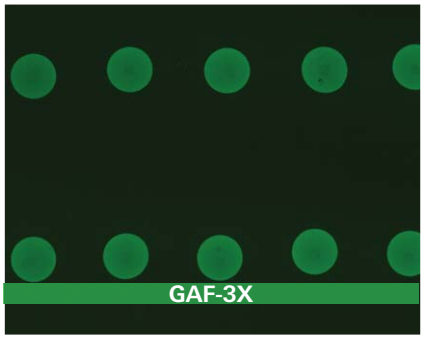

**Liver:** fluorescence of the vessel walls of the intralobular sinusoids. **Oesophagus**: broad fluorescent layer underneath the mucous epithelium, honeycomb-like fluorescence in the lamina muscularis mucosae. **Intestine**: fluorescence of the connective tissue lining the villi and intestinal crypts, and the submucosa endothelia, honeycomb-like fluorescence in the muscular layer.

## Comparison of liver and oesophageal tissues in Anti-Endomysium IIFT

In a study with 298 paediatric coeliac disease patients (bioptically confirmed, > Marsh 2) and 574 controls (including 53 patients with chronic inflammatory bowel diseases), the liver and esophageal substrates were evaluated for sensitivity and specificity using EUROIMMUN Anti-Endomysium IIFT (IgA and IgG).[4] In this study, EmA IgA antibody detection achieved the highest diagnostic accuracy and it was shown that both tissues are equally suitable for the detection of EmA (IgA and IgG). Furthermore, it was found that the interpretation of EmA on liver endomysium is easier compared to oesophageal endomysium due to less interference with other antibodies, such as ASMA.

| Substrate | | IIFT Mosaic | | | |
|---|---|---|---|---|---|
| | | n | IgA | n | IgG |
| Liver | Sensitivity | 298 | 95.6% | 298 | 63.1% |
| | Specificity | 574 | 97.2% | 574 | 96.3% |
| Oesophagus | Sensitivity | 298 | 95.3% | 298 | 52.0% |
| | Specificity | 574 | 98.1% | 574 | 99.5% |

## EUROPLUS Anti-Gliadin (GAF-3X) IIFT

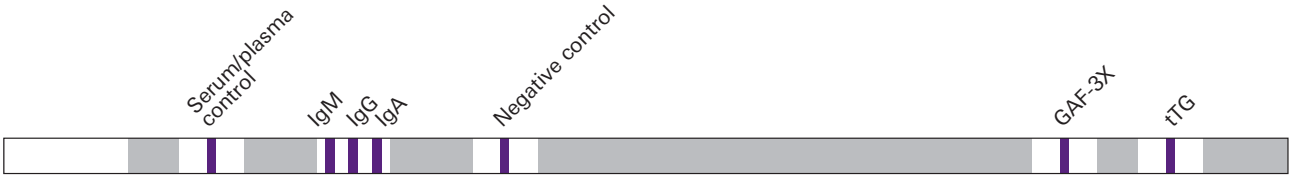



In EUROPLUS immunofluorescence tests, antibody detection is performed using both tissue sections or cell substrates and monospecifically reacting antigen dots. For the EUROPLUS Anti-Gliadin (GAF-3X) IIFT, the designer antigen gliadin (GAF-3X) is coated onto BIOCHIPs in small droplets. In the case of a positive result, the circular substrate spots show a clearly visible fluorescence. The monospecific EUROPLUS substrate is offered in the form of mosaics in combination with tissue substrates for the parallel detection of anti-DGP antibodies and EmA. When the Anti-Gliadin (GAF-3X) ELISA was used as refe-rence method, the EUROPLUS Anti-Gliadin (GAF-3X) IIFT showed a sensitivity of 95% for IgA (n = 122) and of 100% for IgG (n = 114). In a panel of healthy blood donors, a specificity of 99% for IgA (n = 200) and of 100% for IgG (n = 200) was determined.

# III. Immunoblot test systems for the diagnosis of coeliac disease

## EUROLINE Coeliac Disease Profile (IgA, IgG)

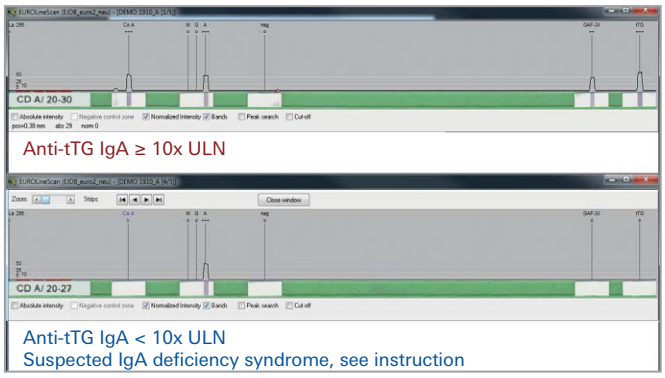
Combining anti-tTG and anti-GAF-3X antibody detection ensures the best diagnostic performance.[5] The combination of tTG and GAF-3X in the EUROLINE Coeliac Disease Profile (IgA, IgG) enables simultaneous detection of reactions to both antigens.




EUROLINE Coeliac Disease Profile

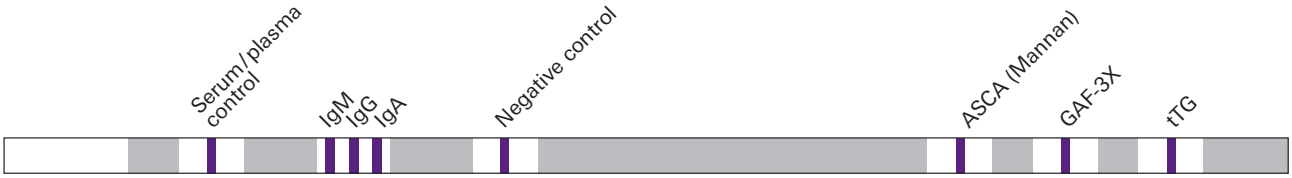
Several serum panels that had been precharacterised by a CE-marked anti-GAF-3X or anti-tTG ELISA reference test, were investigated for anti-GAF-3X and anti-tTG antibodies using the EUROLINE Coeliac Disease Profile. With respect to the ELISA, the EUROLINE yielded a sensitivity of 88.9% for both anti-GAF-3X IgA (n = 45) and anti-GAF-3X IgG (n = 46), at a specificity of 97.0% and 100,0%, respectively. The sensitivity of the EUROLINE for anti-tTG was 100% both for IgA (n = 44) and IgG (n = 44), at a specificity of 96.9% and 100%, respectively. The reference range was determined by investigating a sample panel of healthy blood donors (n = 150). All samples reacted correctly as negative.

If the immunoblot strips are evaluated using the EUROLineScan software, quantitative analysis of the measurement results (determined based on the criterion "upper limit of normal", ULN) is possible. The presence of a result ≥ 10 x ULN is displayed automatically. Moreover, the EUROLINE Coeliac Disease Profile (IgA) includes an IgA-specific serum/plasma control to indicate IgA deficiency, which occurs frequently in patients with coeliac disease. If EUROLineScan detects a positive result for the IgA conjugate control, but a negative signal for the serum/plasma control, the program issues an alert indicating suspected IgA deficiency syndrome. Thereby, the risk of false negatives is effectively prevented.

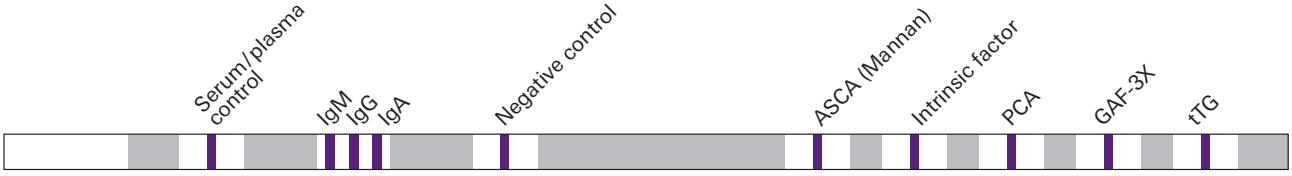



## EUROLINE Autoimmune Gastrointestinal Diseases

In addition to the detection of coealic disease-specific antibodies against tTG and GAF-3X and the exclusion of general IgA deficiency syndrome, the EUROLINE Autoimmune Gastrointestinal Diseases (IgA) enables detection of antibodies against mannan from *Saccharomyces cerevisiae* (ASCA) and thus differentiation of coeliac disease from Crohn's disease. Moreover, the EUROLINE Autoimmune Gastrointestinal Diseases (IgG) allows determination of anti-parietal cell antibodies (PCA) and antibodies against intrinsic factor, which are characteristic of autoimmune gastritis and pernicious anaemia.




EUROLINE Autoimmune Gastrointestinal Diseases (IgA)




EUROLINE Autoimmune Gastrointestinal Diseases (IgG)

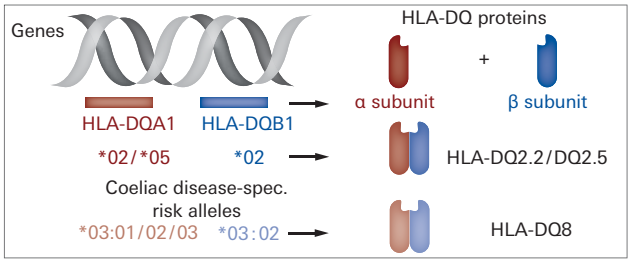
The sensitivity and specificity of the EUROLINE tests were investigated based on several sample panels precharacterised using a CE-marked test. For the detection of anti-tTG antibodies (n = 44) the sensitivity was 100.0%, at a specificity of 100% (IgA) and 96.9% (IgG). The sensitivity of the anti-GAF-3X determination with the EUROLINE amounted to 88.9%, at a specificity of 100% (IgA, n = 45) and 97% (IgG, n = 46). ASCA of class IgA were detected with a sensitivity of 90.9%, at a specificity of 91.3% (n = 49). For ASCA IgG these values were at 100% (n = 30). Antibodies against parietal cells and intrinsic factor were detected with a sensitivity of 100% each (n = 30), at a specificity of 90 and 100%, respectively.

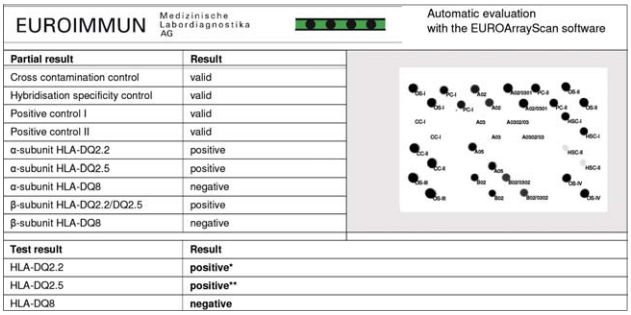
## IV. Molecular genetic determination of the HLA types

## EUROArray HLA-DQ2/DQ8-h Direct

The EUROArray HLA-DQ2/DQ8-h Direct enables simple and fast determination of HLA-DQ types that are clinically relevant in coeliac disease. HLA-DQ molecules are heterodimers composed of an alpha and beta subunit. The alpha and beta subunits are coded by the genes HLA-DQA1 and HLA-DQB1, respectively. In the human population there are a large number of different variants of these genes (alleles). The allele combinations which code for HLA-DQ2.2, -DQ2.5 and -DQ8 are considered risk factors for the development of coeliac disease.

The EUROArray HLA-DQ2/DQ8-h Direct detects all clinically relevant HLA-DQA1 and HLA-DQB1 alleles and allows for an improved risk assessment through the differentiation between the homo- and heterozygous presence of the alleles coding for the alpha and beta subunits of HLA-DQ2.2 and -DQ2.5. In this way, the HLA-DQ2 and HLA-DQ8 types are clearly identified and a secure diagnosis is enabled. If neither of the two types is detected in a patient, coeliac disease may be excluded with a probability of nearly 100 % (negative predictive value at least 98 %). The test can be easily performed using EDTA blood or isolated genomic patient DNA as sample material. Evaluation, reporting, and data archiving for the system are performed objectively and automatically by means of the EUROArrayScan software.

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Automatic evaluation with the EUROArrayScan software

| Partial result | Result |
|---|---|
| Cross contamination control | valid |
| Hybridisation specificity control | valid |
| Positive control I | valid |
| Positive control II | valid |
| α-subunit HLA-DQ2.2 | positive |
| α-subunit HLA-DQ2.5 | positive |
| α-subunit HLA-DQ8 | negative |
| β-subunit HLA-DQ2.2/DQ2.5 | positive |
| β-subunit HLA-DQ8 | negative |

| Test result | Result |
|---|---|
| HLA-DQ2.2 | positive* |
| HLA-DQ2.5 | positive** |
| HLA-DQ8 | negative |

# EUROIMMUN test systems for coeliac disease diagnostics in application

## Secure diagnosis without biopsy

- In a study with 1071 tested samples, the Anti-tTG ELISA (IgA) contributed to a clear diagnosis. The additional determination of anti-DGP antibodies by means of the Anti-Gliadin (GAF-3X) ELISA (IgG) further increased the statistical accuracy. Thus, the share of tested persons requiring biopsy could be reduced to below 11 %.[6]
- The combination of the Anti-tTG ELISA (IgA) and the Anti-Gliadin (GAF-3X) ELISA (IgG) is best diagnostic option to confirm and exclude coeliac disease. The first international prospective study with 898 patients proved that a large number of biopsies could be prevented with this strategy.[5]
- The value of the diagnostic criterion "anti-tTG IgA ≥ 10 x ULN" was confirmed in an international multicentre study with 707 paediatric patients. Taking into account this criterion helped to prevent more than 50 % of biopsies.[7]
- In comparison with tests from competitors, the Anti-tTG ELISA (IgA) was able to detect the most ≥ 10 x ULN results in a panel of 59 bioptically confirmed patients.[8]

| n = 59 | ≥ 10 x ULN (IgA) |
|---|---|
| Anti-tTG ELISA | 53 |
| Competitor 1 | 45 |
| Competitor 2 | 42 |
| Competitor 3 | 27 |

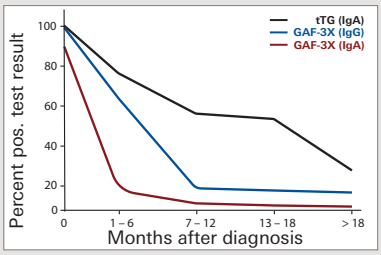
## Reliable serology in patients with IgA deficiency

- The detection of GAF-3X-specific, anti-DGP antibodies (IgG) is a useful alternative to IgA-specific tests in patients with IgA deficiency syndrome.[5]
- The sensitivity of the Anti-Gliadin (GAF-3X) ELISA (IgG) proved to be significantly higher compared to the sensitivity of the EmA IIFT (IgG) in a panel of 34 patients with selective immunoglobulin A deficiency (sIgAD), and exceeds the sensitivity of the Anti-tTG ELISA (IgG).[9]
- In contrast to the clinical sensitivity of the Anti-EmA IIFT (IgG) in panels with normal sIgAD prevalence ([5,] cf. page 6), an increased sensitivity of the Anti-tTG ELISA (IgG) is observed in the isolated investigation of a pure sIgAD panel.[9]

| n = 34 | Sensitivity | Specificity |
|---|---|---|
| Anti-GAF3X ELISA (IgG) | 88.2 % | 97.5 % |
| Anti-tTG ELISA (IgG) | 82.4 % | 99.9 % |
| EmA IIFT (IgG) | 75.8 % | 99.0 % |

## Coeliac disease-associated antibodies correlate with adherence to gluten-free diet

- A gluten-free diet (GFD) is essential for the health of patients with coeliac disease. In an 18-month study, the majority of 78 tested patients adhering to GFD showed a significant decrease in coeliac disease-associated IgA and IgG antibodies. Moreover, in comparison to tests from other manufacturers, the EUROIMMUN Anti-tTG ELISA (IgA) reacted most sensitively to increasing titers if the dietary rules were not observed.[8]



## Order information

| Test system | Testname | Substrate | Order number |
|---|---|---|---|
| **ELISA** | Anti-Tissue Transglutaminase ELISA | Tissue Transglutaminase (tTG) | EA 1910-9601 A or G |
| | Anti-Gliadin (GAF-3X) ELISA | Gliadin (GAF-3X) | EV 3011-9601 A or G |
| **IIFT** | IIFT Primate Oesophagus | Endomysium | FA 1911-#### A or G |
| | IIFT Primate Intestine | | FA 1913-#### A |
| | IIFT Primate liver | | FA 1914-#### A or G |
| | EUROPLUS Gliadin (GAF-3X) with primate oesophagus | Gliadin (GAF-3X) | FA 1911-####-1 A |
| | EUROPLUS Gliadin (GAF-3X) with primate intestine | | FA 1913-####-1 A |
| | EUROPLUS Gliadin (GAF-3X) with primate liver | | FA 1914-####-1 A or G |
| **EUROLINE** | EUROLINE Coeliac Disease Profile | tTG/gliadin (GAF-3X) | DL 1910-1601 A or G |
| | Autoimmune Gastrointestinal Diseases (IgA) | tTG, GAF-3X, mannan | DL 1360-#### A |
| | Autoimmune Gastrointestinal Diseases (IgG) | tTG, GAF-3X, PCA, intrinsic factor, mannan | DL 1360-#### G |
| **EUROArray** | EUROArray HLA-DQ2/DQ8-h Direct | DNA microarray | MN 5320-####-V |

## References

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